Pathologic Aminoaciduria in Experimental Ascorbic Acid Deficiency. (30481)

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Pathologic aminoaciduria, defined as excessive urinary excretion of free amino acids, has been described in numerous inherited and acquired metabolic abnormalities in man (1-3), including ascorbic acid deficiency(4-6). Aminoaciduria has also been produced in animals by intoxication with maleic acid(7,8) galactose(9) and phenylalanine(10). The present studies were designed to determine if experimental ascorbic acid depletion in the guinea pig can also serve as an animal model with which to study the physiologic and biochemical derangements leading to pathologic aminoaciduria.

Materials and methods. Twelve 21-day-old male guinea pigs of the N.I.H. strain, weighing approximately 200 g each, were placed in individual metabolic cages which allowed separation of urine and feces. For one week all animals were fed an ascorbic acid deficient diet* supplemented with sufficient L-ascorbic acid* (8 mg per g diet) to insure ample intake of this vitamin(11). At the end of the adjustment period the animals were divided into 3 groups. One group (normal *ad libitum*) was fed the ascorbic acid deficient diet *ad libitum* supplemented as above with L-ascorbic acid. The second group (ascorbic acid deficient) was fed the same diet *ad libitum* without addition of ascorbic acid. The third group (normal pair-fed) was paired with the ascorbic acid deficient group and received the ascorbic acid supplemented diet equal in weight to that which had been consumed by the paired mate the previous day.

The diet was fed as a wet paste to avoid spillage into the metabolic cage. Feeding cups were replenished 3 times daily from individual refrigerated supplies. Food consumption and body weight were recorded daily.

Seven 48-hour urine collections were obtained at intervals throughout a 23-day study. The urine samples were collected in glass vessels containing 2 ml toluene to prevent bacterial contamination and were stored frozen. Collection periods for the pair-fed group followed the normal group fed *ad libitum* and the ascorbic acid deficient group by 24 hours. Urine samples were analyzed for free alpha amino nitrogen by the method of Rubinstein and Pryce(12). Quantitative determination of individual free amino acids in selected urine samples from each group of animals was undertaken using an amino acid analyzer as described by Spackman, Stein and Moore(13).

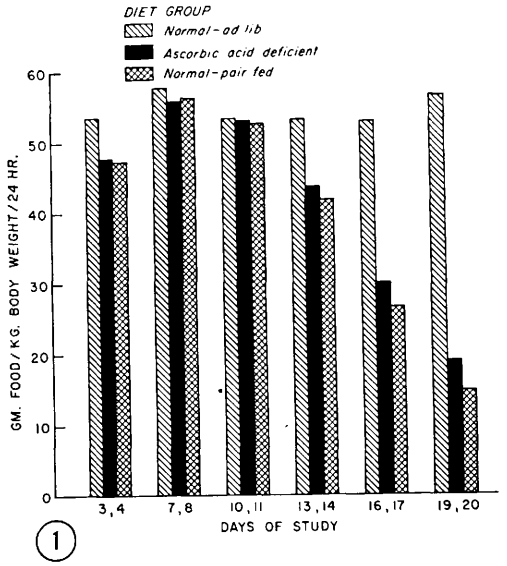
After terminating the experiment, skeletal X-rays and gross and microscopic anatomic findings were obtained in each group of animals.

Results. Food intake and body weight. In the normal group fed *ad libitum,* food intake and body weight increased throughout the study (Fig. 1 and 2). Food intake and weight gain in the ascorbic acid deficient animals were comparable to those in controls until day 14 when both food intake and body weight began to fall. The intake of the pair-fed groups was limited by experimental design, but, in contrast to the ascorbic acid depleted group, weight gain continued after day 14 (Fig. 2), albeit at a rate slightly less than in the normal group fed *ad libitum.* Coincident with the fall in intake and body weight, the ascorbic acid deficient animals exhibited listlessness, sensitivity to touch and difficulty in walking, signs characteristic of experimental scurvy.

Urinary amino acid excretion. As noted in Fig. 3, urinary alpha amino nitrogen rose slightly during the study in the normal group fed *ad libitum.* This rise was small, however, compared to that seen in the ascorbic acid deficient groups. By day 10 these animals excreted significantly greater quantities of free amino acids than either the *ad libitum*

* Obtained from Nutritional Biochemicals Corp.

or pair-fed control animals, and by day 14 the ascorbic acid deficient group excreted 3-4 times as much alpha amino nitrogen. This aminoaciduria cannot be attributed to starvation since it appeared prior to the reduction in food intake and was not observed in the pair-fed group. When amino acid excretion was corrected both for food intake and body weight, the marked elevation of alpha amino nitrogen in the ascorbic acid deficient animals from day 14 on, was striking compared to the relatively constant values noted in both control groups. Column chromatographic analyses of urine samples from each group on days 16-17 revealed that the aminoaciduria observed in the ascorbic acid deficiency was due predominantly to a marked elevation of glycine and to less striking increases in asparagine and glutamine.



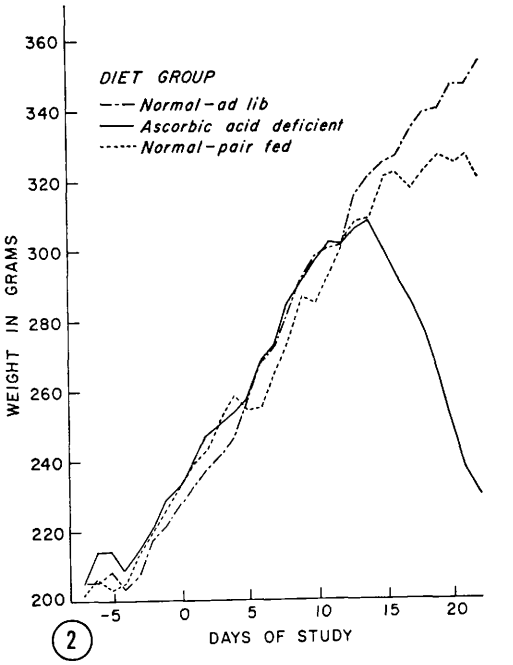


FIG. 1. Influence of ascorbic acid depletion on food intake in the guinea pig.

FIG. 2. Effect of ascorbic acid depletion on body weight.

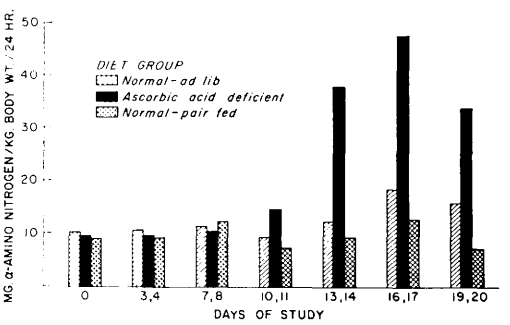


FIG. 3. Pathologic aminoaciduria produced by ascorbic acid depletion. A statistically significant ($P<.01$) increase in alpha amino nitrogen was noted in the ascorbic acid-deficient animals from days 10 and 11 to the end of the study.

Urinary pH, glucose and phosphate. In addition to amino acid analyses, urine samples were analyzed for pH, glucose and phosphate. The ascorbic acid deficient animals exhibited no glycosuria nor was the pH of their urine different from that of the controls. Urinary phosphate rose in the ascorbic acid deficient guinea pigs from day 14 on, but this finding was also noted in the pair-fed controls, and hence must be attributed to starvation rather than to ascorbic acid deficiency *per se*.

Post mortem examination. The ascorbic acid deficient animals exhibited numerous pathologic findings characteristic of scurvy including: deformity, swelling and fracture of the wrist and knee joints; rectal and intramuscular hemorrhages; enlarged adrenal glands; lack of calcification of skeletal osteoid; and cellular disorganization in bone. None of these findings was present in either control group.

Discussion. The present studies demon-

strate that guinea pigs maintained on an ascorbic acid deficient diet exhibit prominent aminoaciduria which cannot be attributed to starvation or weight loss. Chromatographic analysis suggests that the observed increase in urinary alpha amino nitrogen is due largely to excessive loss of glycine. Schoenheyder and Lyngbye(14) reported depressed concentrations of glycine in plasma and muscle in ascorbic acid deficient guinea pigs, suggesting that the elevated urinary glycine results, at least in part, from faulty renal tubular absorption of this amino acid. These findings are in agreement with those of Huisman and Jonxis(4,5), who reported that the aminoaciduria observed in ascorbic acid deficient children was associated with normal plasma levels of free amino acids. The present studies indicate that the aminoaciduria of ascorbic acid deficiency has distinct specificity since neither glycosuria nor phosphaturia, hallmarks of generalized proximal tubular abnormalities, were found.

The cellular mechanisms responsible for the observed aminoaciduria have not been clarified. It is generally assumed that the transport processes by which amino acids enter tissue cells are energy dependent and are mediated by specific sites or "carriers" in the cell membrane. Hence, in the most general sense, interference with transport processes could result from inhibition or uncoupling of energy-yielding processes needed for "active" transport; or from alteration of the specific interaction between the substrate amino acid and the membrane carrier site. The present results suggest that future *in vitro* studies of transport mechanisms in tissues of ascorbic acid deficient animals would be of value in clarifying the mode of action of ascorbic acid on transport mechanisms.

Summary. Ascorbic acid deficiency in the guinea pig is associated with prominent aminoaciduria, due largely to excessive glycine excretion. Pair-feeding studies indicate that this aminoaciduria results from ascorbic acid depletion *per se,* and cannot be attributed to starvation or weight loss. It is concluded that experimental scurvy provides a suitable model for future *in vivo* or *in vitro* studies of amino acid transport.

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